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PRINCIPAL INVESTIGATOR: ~~Dr. John R. Anderson~~*

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14. ABSTRACT. There are two signaling pathways: HIF-1 α and Stat3 are important targets for prostate cancer (PC) therapy. HIF-1 α is the key gene that controls the amount of the transcription of hypoxia-inducible genes. Overexpression of HIF-1 α not only strongly enhances the rate of tumor growth and metastatic potential, but also contributes to resistance to radiotherapy and chemotherapy, leading to treatment failure and increased patient mortality. Stat3 participates in oncogenesis through the upregulation of genes encoding apoptosis inhibitors, cell-cycle regulators, and inducers of angiogenesis in many human cancers, including prostate cancer. Also, Stat3 suppresses anti-tumor immune responses and mediates the cancer-promoting properties. Previously, we demonstrated that treatment of PC in preclinical models with a combination of a Stat3 inhibitor and a HIF-1 α inhibitor greatly enhanced drug efficacy and dramatically increased apoptosis in human tumors compared with the use of either agent alone, showing that targeting both Stat3 and HIF-1 α together can improve tumor response. In this research period, we have developed TEL03, a dual inhibitor from nature products, for cancer therapy. TEL03 targets both HIF-1 α and Stat3, blocks the expression of their down-regulated oncogenes and significantly suppresses tumor growth *in vivo*. TEL03 also demonstrated a greater-than-expected *in vivo* potency, perhaps because of synergy arising from the ability of this agent to simultaneously target two critical oncogenic pathways.

15. SUBJECT TERMS

Stat3, HIF-1alpha, TEL03, A dual inhibitor, cancer therapy

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INTRODUCTION

A1. Project title: A novel dual Inhibitor that targets HIF-1 α and Stat3 for cancer therapy

A2. Significance. Two pathways: Stat3 (signal transducer and activator of transcription 3) and HIF-1 (hypoxia-inducible factor-1) have been demonstrated to be important targets for cancer therapy (1-4). Stat3 exist as monomers or N-terminal head-to-head dimers in the cytoplasm (5-7). When stimulated by cytokines or growth factors, such as JAK, Scr or EGFR (8, 9), Stat3 is activated upon phosphorylation on tyrosine residue Y705 (10). Tyrosine phosphorylation induces formation of a parallel dimer through their SH2 domains (5, 7). The activated dimers translocate to the nucleus, where they bind to DNA-response elements in the promoters of target genes and activates transcription. Stat3 participates in oncogenesis through the upregulation of genes encoding anti-apoptosis (Bcl-x_L, Bcl-2, Mcl-1, and survivin), cell-cycle regulators (cyclin D1 and *c-myc*), and inducers of angiogenesis (VEGF) (1, 11, 12). Also, immune system plays a crucial role in controlling tumor incidence and growth. Stat3 signaling is a major intrinsic pathway of cancer inflammation and mediates the cancer-promoting properties. Stat3 suppresses anti-tumor immune responses and promotes inflammation-induced cancer, making it an attractive target (13, 14).

HIF-1 protein is a heterodimer consisting of two subunits: HIF-1 α and HIF-1 β . Under normoxia, prolyl hydroxylases (PHDs) hydroxylate the prolyl residues of HIF-1 α at amino acids P402 and P564, which are then recognized by VHL (Von Hippel-Lindau) and targeted to the ubiquitin proteasome pathway. An additional hydroxylation at N803 blocks the binding of p300 and CBP (Crel-binding protein) to HIF-1 α and inhibits HIF-1-mediated gene transcription. Under hypoxia, HIF-1 α is not hydroxylated and not degraded. The unmodified protein then dimerizes with HIF-1 β . As N803 is not hydroxylated, p300 (or CBP) can bind to HIF-1 α , allowing transcriptional activation of HIF-1 target genes, which are included more than 70 putative hypoxia-inducible genes, to date, and involved in many cell processes including glucose metabolism, erythropoiesis, angiogenesis, anti-apoptosis, metastasis, and other functions (15-18). HIF-1 α was demonstrated to overexpress in many human cancers, including colon, breast, gastric, lung, and skin, ovarian, prostate, renal and pancreatic carcinomas (19-21). Overexpression of HIF-1 α not only strongly enhances the tumor growth rate and metastatic potential, but also contributes to resistance to radiotherapy and chemotherapy, leading to treatment failure and increase in patient mortality (22, 23).

A3. Hypothesis and Innovation. Drug development has moved into targeting molecules based on their important biological function in cancer (24), which may be considered one of the most remarkable developments in the field of cancer research and therapeutics in past decades (25). A selective inhibition of a target molecule dramatically enhances therapeutic efficacy; however, it also can result in unexpected consequences, e.g. rapid development of resistance as a single molecule is inhibited with high selectivity; drug becomes less effective or drug responsibility is reduced after prolonged use; reduced delivery of an anticancer agent to all regions of the tumor; and a change in micro-environment that reduces drug response (24, 26). It is becoming increasingly evident that increasing the cure rate of many cancers will require combinations of drugs active against more than one validated tumor target. Our previous studies demonstrated (27) that compared with targeting Stat3 and HIF-1 α alone, the treatment using two inhibitors to target Stat3 and HIF-1 α simultaneously greatly increased drug efficacy and dramatically induced apoptosis in prostate tumors.

As a novel strategy, developing an anti-cancer agent that can target dual onco-molecules for cancer therapy is proposed here. Recently, we have explored a perylene derivative TEL03 (28) to be a dual anti-cancer agent that targets both HIF-1 α and Stat3, significantly inhibits activations of HIF-1 α and p-Stat3, and blocks the expression of their down-regulated oncogenes (e.g. Bcl2, VEGF, Glut1, and others) in cancer cells. TEL03 also can significantly suppress the growth of human tumors in xenograft models, showing that it has great potential to be a potent anti-cancer agent.

BODY AND KEY RESEARCH ACCOMPLISHMENTS

1. Screen for novel inhibitors. Two pathways: Stat3 and HIF-1 have been demonstrated to be important targets for cancer therapy. Here we have screened many drug candidates, which were derived from Chinese medicinal herbs or synthesized by chemistry, including *Zanthoxylum nitidum* (NDT) and derivatives, *Protostephanine* (CEP), *Tinospora Root* (JTH), *Radix Steohanian Tetrandrae* (BLT), *Chelerythrine* (CLT), and a perylene derivative (TEL03) (Fig.1A). The results demonstrated that the molecules, NDT (#2), TEL03 (#5) and TEL03+HCL (#12), have ability to inhibit HIF-1 α expression in cancer cells (MDA-MB-231) under hypoxia (Fig.1B). Meantime, TEL03 and TEL03+HCL also showed the ability to inhibit phosphorylated Stat3 (p-Stat3) expression without blocking total Stat3 (T-Stat3), which is mainly composed of unphosphorylated Stat3 (Fig.1C). Thus, TEL03 was selected to be a candidate of anti-cancer agent for further tests.

2. Discovery of a dual inhibitor that targets both Stat3 and HIF-1 α . All of TELs (TEL01-10) were originally designed to target G-quadruplex DNA (or RNA) in telomere (29,30). We have performed immunoblotting assay in human cancer cells, including breast, pancreatic, ovarian and other cancer cells, to screen all the TELs. Only TEL03 was observed to inhibit both HIF1 α and p-Stat3 simultaneously. IC50s of TEL03 are 3~6 μ M in cancer cells. TEL03 also suppresses the expression of the HIF-1 α or Stat3 regulated proteins Bcl2 under normoxia and hypoxia. Importantly, TEL03 does not inhibit JCK, SCR, p-Stat1, p-Stat5, and total Stat3 (T-Stat3) in STAT signaling and does not inhibit p300 (a co-activator of HIF-1 α), HIF-1 β (ARNT), p-ERK and ERK (upstream proteins in HIF-1 signaling) as well (Fig.2), showing that TEL03 has a special mechanism to target HIF-1 α and Stat3 but other TELs do not have.

3. TEL03 inhibits Stat3 and HIF-1 α independently. The previous study proposed that targeting Stat3 with a small-molecules inhibitor also could block the expression of HIF-1 α in cancer cells (31). To identify whether TEL03 inhibits HIF-1 α through the inhibition of Stat3 or TEL03 directly inhibits HIF-1 α , we performed two assays: (1) first, using T40214

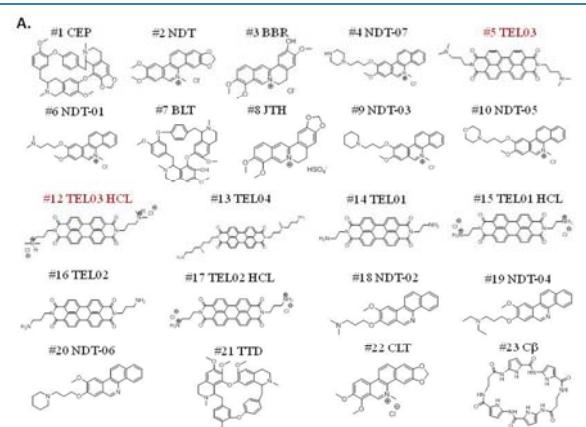


Fig. 1A: The molecules were screened

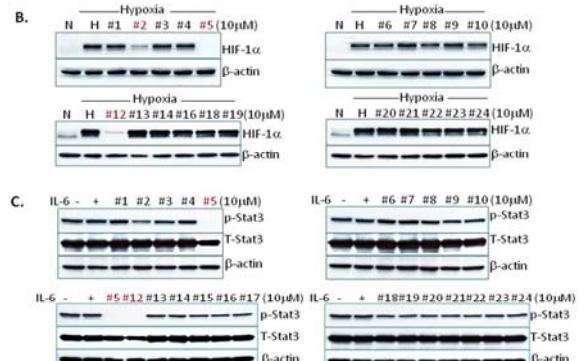


Fig. 1B-C: The western blots for drug screen showed that TEL (#5) (or TEL+HCL (#12)) has the ability to inhibit the expressions of both HIF-1 α and phosphorylated Stat3 (p-Stat3) under hypoxia and normoxia (N means: normoxia).

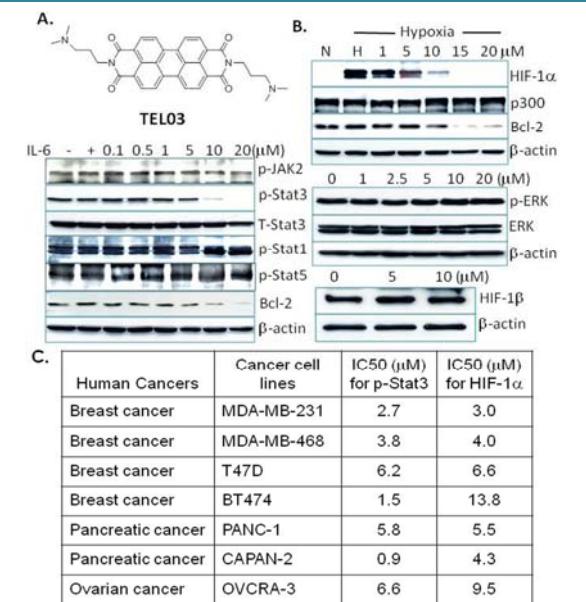


Fig. 2: (A) TEL03; (B) TEL03 inhibits Stat3 and HIF-1 α under normoxia and hypoxia; (C) IC50s of TEL03.

(32,33), a developed Stat3 inhibitor, to inhibits p-Stat3 in cancer cells (PANC-1) and then after 3 hours adding TEL03 to detect whether TEL03 can inhibit HIF-1 α in the sample without p-Stat3; and (2) performing qRT-PCR to measure RNA levels of HIF-1 α since if TEL03 inhibits HIF-1 α activity through inhibition of Stat3, TEL03 should strongly suppress the RNA expression of HIF-1 α . Fig.3A showed that T40214 totally inhibited p-Stat3 activity (lanes 4&5) but did not inhibit T-Stat3 and HIF-1 α (lane4). HIF-1 α was inhibited after adding TEL03 (lane 5), showing that TEL03 inhibits HIF-1 α expression in the absent of p-Stat3. Also, the PCR data demonstrated that TEL03 does not suppress the RNA level of HIF-1 α in cancer cells (Fig.3B). The results provided evidence that TEL03 inhibits Stat3 and HIF-1 α , independently, and TEL03 directly targets HIF-1 α protein in hypoxic cells.

4. The mechanism of TEL03 Targeting Stat3. The western blot of the time-dependent inhibition of Stat3 demonstrated (Fig.4A) that TEL03 significantly inhibited phosphorylated Stat3 (p-Stat3) activity in 30 mins but did not inhibit T-Stat3, JAK2, and SRC kinases within 6 hours. This result suggested that TEL03 specially inhibits p-Stat3 by blocking Stat3 phosphorylation. SPR (surface plasmon resonance)-based binding assays can determine whether TEL03 has the ability to block Stat3 binding to a ligand in EGFR for phosphorylation. Previous studies identified that the key residues in Stat3 SH2 domain, which bind the pYxxQ-ligand motif of EGFR, include K591, R609, S611, E612, S613, E638, and Y640 (34,35). First, biotinylated pYxxQ-ligand motif (LPVPE(pY)INQSVLP) was immobilized on a streptavidin coated sensor chip (35,36). Adding Stat3 alone and Stat3/TEL03 complex into the chip, the results shows that TEL03 dramatically reduced the binding of Stat3 to the peptide, suggesting that TEL03 directly binds to Stat3 SH2 domain and blocks Stat3 binding to the peptide ligand (Fig.4B). The modeling structure of TEL03/Stat3 complex predicted that TEL03 strongly interacts with the residues of E612, S613 and E638 in SH2 domain and is capable of disrupting the interaction between Stat3 and its phosphotyrosyl peptide ligand (Fig.4C).

5. The mechanism of TEL03 inhibition of HIF-1 α . (1) A GST pull-down assay was employed to determine the whether TEL03 specifically interacts with HIF-1 α protein. After bead pull down, the samples were loaded onto a polyacrylamide gel. Fig.5A shows that p300 did not bind with GST

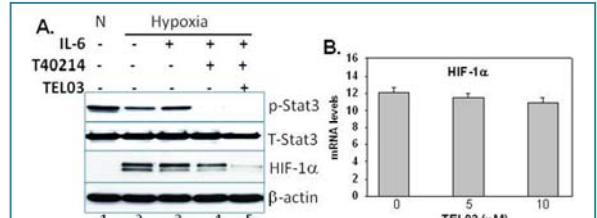


Fig.3: (A) Western blot data (N: normoxia); (B) qRT-PCR data in PANC-1 cells.

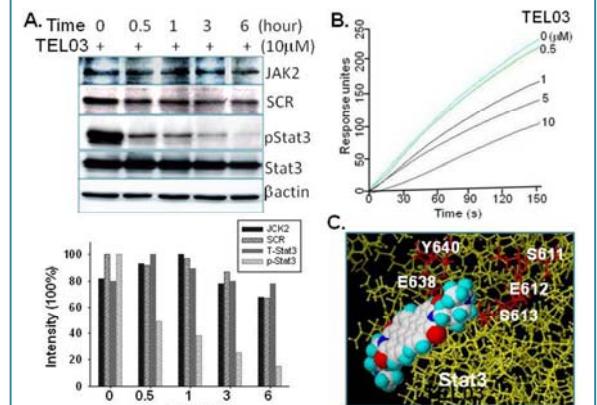


Fig.4: (A) Time-dependent western blot; (B) SPR data. (C) Molecular modeling shows that TEL03 bind within SH2 domain of Stat3 and disrupt the Stat3 phosphorylation.

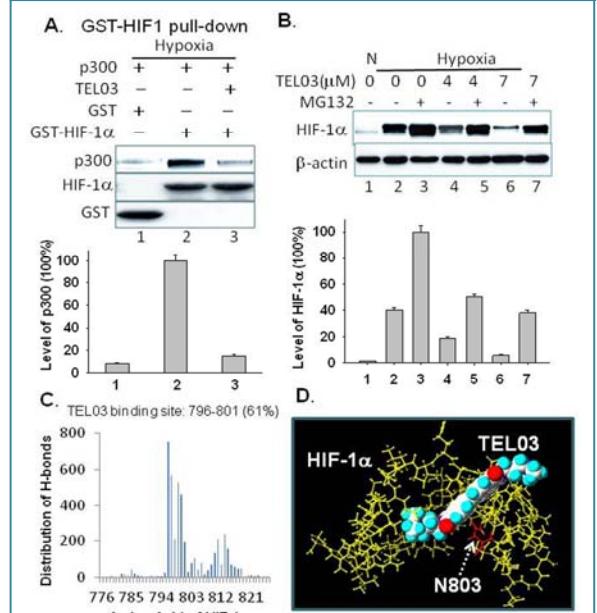


Fig.5: (A) The pull-down data (see details in text). (B) Western data shows HIF-1 α degradation (see details in text). (C) The distribution of H-bonds that formed between TEL03 and the C-terminal domain of HIF-1 α . (D) The structure of TEL03 in HIF-1 α .

(lane1). The samples of GST-HIF-1 α /p300 and GST-HIF-1 α /p300+TEL03 have an equal level of HIF-1 α proteins; however, the level of p300 in lane 3 is much less than that in lane 2. These results clearly demonstrated that p300 strongly binds with HIF-1 α in hypoxia (lane2) and TEL03 directly interacts with HIF-1 α protein and blocks the binding interaction between HIF-1 α and p300 (lane3). (2) To determine whether TEL03 promotes the proteasomal degradation of HIF-1 α in hypoxic cells, cancer cells (MDA-MB-468) were exposed to TEL03 alone or TEL03 plus MG132, which is an inhibitor of proteasome, at 1% O₂ for 18 hrs. Comparing the hypoxia-induced levels of HIF-1 α with MG132 (proteasome blocked) and without MG132 (proteasome activated), the results showed that TEL03 greatly induces the degradation of HIF-1 α through the proteasome in hypoxic cells (Fig.5B). (3) To gain insight into the molecular interaction between TEL03 and HIF-1 α , we randomly docked each TEL03 1000 times onto the C-terminal domain of HIF-1 α (37) without setting any constraints and then analyzed the distribution of hydrogen (H) bonds formed between each TEL03 and HIF-1 α because H-bonds play an important role in governing the interaction between HIF-1 α and TEL03. The analysis showed that the hydrogen bonds formed between each TEL03 and the C-terminal domain of HIF-1 α were highly concentrated in the region of amino acids 796 to 801 (61%) (Fig.5C). Binding energy between C-terminal of HIF-1 α and TEL03 is about -52 kcal, leading to the stable binding complex of HIF-1 α and TEL03 (Fig.5D). This structure demonstrates that TEL03 binds in the range of residues 796 to 801 of HIF-1 α and blocks the interaction between p300 and N803 of HIF-1 α in hypoxia, consistent with the observation in Fig.5A.

6. **TEL03 inhibits VEGF, GLUT1 and induces apoptosis.** Expression of VEGF, which is a key stimulator of angiogenesis, and GLUT1, which can increase intracellular glucose uptake, are hypoxia-upregulated by HIF-1-dependent transcriptional activation (38). Quantitative RT-PCR showed that TEL03 significantly suppressed the expressions of VEGF and GLUT1 mRNA in responding to hypoxia (Fig.6A). MTT was also employed to measure the cell death induced by the effects of TEL03 in cancer cells (MDA-MB-468 and MDA-MB-231) and breast epithelial cell (MCF10A). The data were collected at 48 hours after adding TEL03 in cells. The results showed (Fig.6B) that TEL03 significantly induced apoptosis in the cancer cells; however, TEL03 did not induce cell death in normal cells (MCF10A) at the same condition, suggesting that TEL03 could have a favorable safety profile.

7. **Summary.** TEL03 was demonstrated to have a special mechanism to inhibit Stat3 and HIF-1 α . (1) TEL03 binds with the residues E612, S613 and E638 in the SH2 domain of Stat3, blocks Stat3 tyrosine phosphorylation, and inhibits Stat3 transcriptional activity, leading to decreased cancer cell proliferation, increased apoptosis, and reduced angiogenesis (Fig.7A). (2) TEL03 binds to the region of residues 709 to 801 of HIF-1 α , disrupts the interaction between p300 (co-activator) and residue N803 of HIF-1 α , promotes the proteasomal degradation of HIF-1 α , interrupts HIF-1 α transcriptional activity, and greatly reduces the levels of hypoxia-regulated genes, including VEGF, GLUT1, Bcl2 and others (Fig.7B).

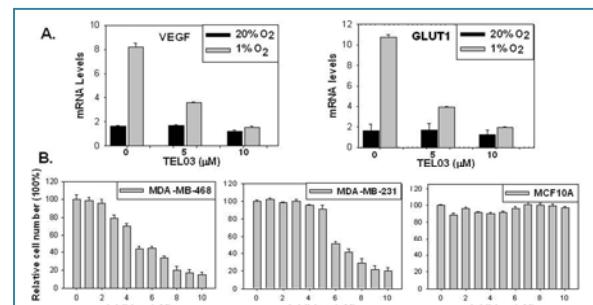


Fig.6: (A) The RNA levels of VEGF and GLUT1 showed that TEL03 significantly suppressed the expression of VEGF and GLUT1 mRNA in responding to hypoxia. (B) MTT results of TEL03 in the cancer cells and in normal cells at the same condition.

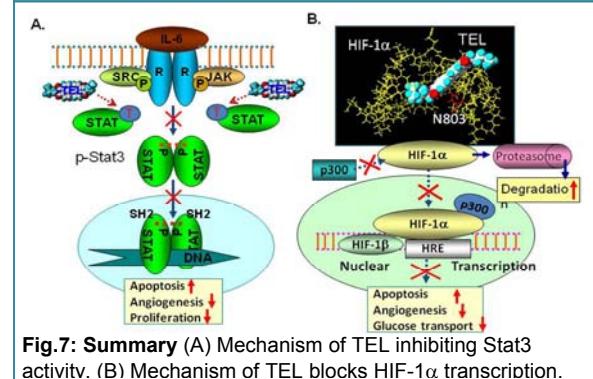


Fig.7: Summary (A) Mechanism of TEL inhibiting Stat3 activity. (B) Mechanism of TEL blocks HIF-1 α transcription.

8. In vivo drug efficacy of TEL03. *In vivo* drug activity will provide important evidence for determining whether TEL03 could be a promising drug candidate. First, pancreatic cancer cells (PANC1) were injected into the right flank of each nude mouse. When the tumors grew over 200mm³, mice were randomly assigned to 2 groups (each group has seven mice): Group 1 was used as controls without treatment; Group 2 was treated by TEL03. We gave 2mg/kg of TEL03 to each mouse in Group 2 every other day for 10 days through IP injection, and then monitored the tumor growth without treatment for another 9 days. Over 19 days the mean tumor volume in untreated mice increased 3.4-fold from 225 to 754 mm³. In contrast, the mean tumor volume in TEL03-treated mice was decreased 3.3-fold from 246 to 75 mm³. The mean tumor weight of untreated mice was 0.5 ± 0.05 g whereas the mean tumor weight of TEL03-treated mice was 0.06 ± 0.01 g (Fig.8 & 9A). After given with 2mg/kg of TEL03 in 6 times (red arrows), the mean tumor volume of pancreatic tumors was dramatically suppressed and the tumors did not re-grow after stopping treatment in 9 days. The body weight of the treated mice was not affected by TEL03 treatment (Fig.9B). The results demonstrated that TEL03 can significantly suppress human tumor growth. TEL03 also showed a greater-than-expected *in vivo* potency, perhaps because of synergy arising from the ability of this agent to simultaneously target two critical oncogenic pathways.

CONCLUSION

In this report, we summarize our development of TEL03, a novel single agent that targets both HIF-1 α and Stat3, blocking the transcriptional activity in each pathway thereby promoting cancer cell apoptosis and suppressing tumor angiogenesis. TEL03 activity was selective within the JAK/STAT pathway since it only blocked Stat3 phosphorylation; it did not reduce levels of p-Stat1 or p-Stat5 and did not inhibit either JAK or Scr kinases. Our modeling and experimental results revealed that TEL03 interacts with residues E612, S613 and E638 within the SH2 domain of Stat3, blocks Stat3 tyrosine phosphorylation, and inhibits Stat3 transcriptional activity, leading to decreased cancer cell proliferation, increased apoptosis, and reduced angiogenesis. In addition, TEL03 inhibited HIF-1 α /2 α but did not target p300 and HIF-1 β . Our experimental and modeling studies also exposed that TEL03 binds to the C-terminus of HIF-1 α within the region of residues 709 to 801. TEL03 binding to HIF-1 α at this site, reduces the interaction between p300 and residue N803 of HIF-1 α and promotes the proteasomal degradation of HIF-1 α , interrupts HIF-1 α transcriptional activity, and greatly reduces the levels of hypoxia-regulated genes, including VEGF, GLUT1 and others. TEL03 did not target p-ERK and ERK, oncoproteins in the MEK/ERK pathway up-stream of HIF-1 α . Thus, TEL03 is a compound that selectively inhibits two critical pathways in oncogenesis, Stat3 and HIF-1 α . Our *in vivo* study showed that treatment of mice bearing pancreatic tumor xenografts (mean size ~ 240 mm³) with TEL03 (2mg/kg) resulted in inhibition of tumor growth, tumor regression, and a marked delay in tumor re-growth. Thus, an agent such as TEL03 capable of targeting both HIF-1 α and Stat3 demonstrated greater-than-expected *in vivo* potency, perhaps because of

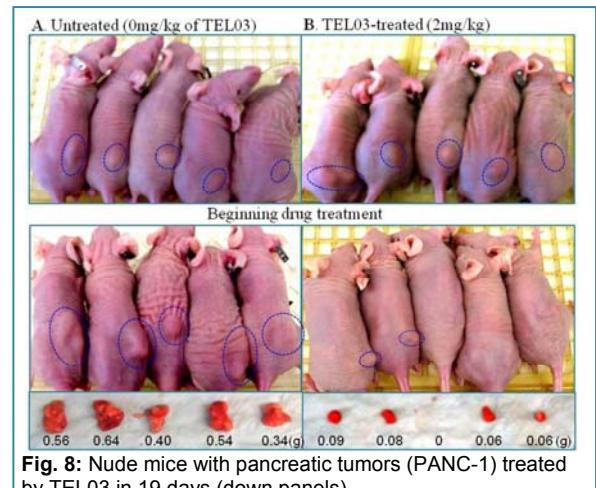


Fig. 8: Nude mice with pancreatic tumors (PANC-1) treated by TEL03 in 19 days (down panels).

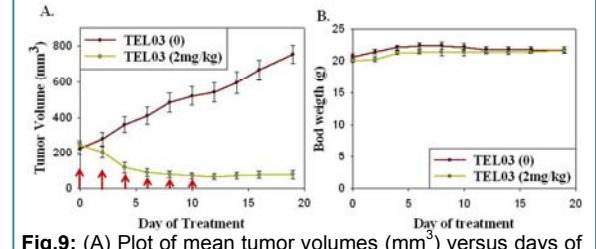


Fig.9: (A) Plot of mean tumor volumes (mm³) versus days of drug treatment. (B) Plot of mean body weight (g) versus days of drug treatment.

synergy arising from the ability of this agent to simultaneously target two critical oncogenic pathways. Targeting both HIF-1 α and Stat3 is considered to be a promising strategy for cancer therapy, including breast, prostate, pancreatic, ovarian and other cancers. TEL03 is considered to be a promising anti-cancer agent and is worth for future study.

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REPORTABLE OUT COMES.

1. Patent application.

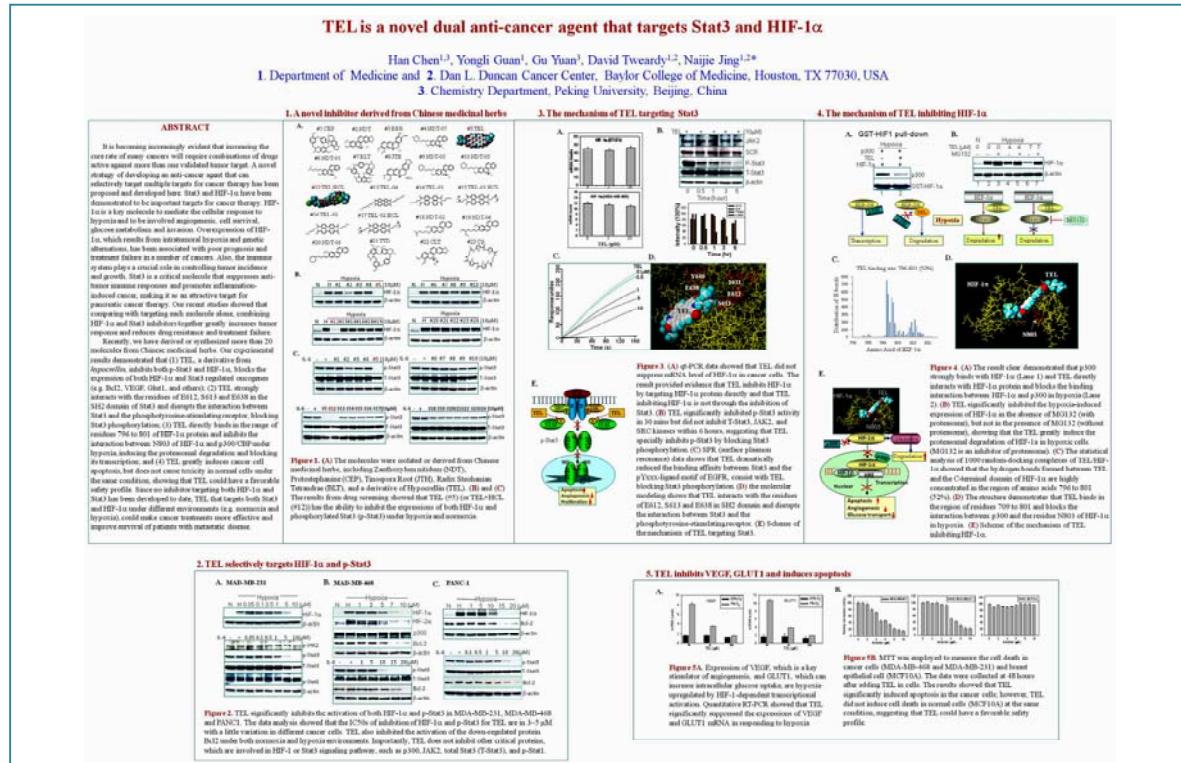
Chinese patent (pending, 2012): Perking University, Jing N (BCM), Guan Y (BCM). “二萘嵌苯衍生物作为p-STAT3/Hif1 α 信号传导通路抑制剂在相关疾病中的应用” (application of perylene derivatives that inhibit p-Stat3/HIF-1 α in medical diseases) (the contributions of Baylor College of Medicine (BCM), USA, and Perking University, China, are 50:50).

2. Publication.

Chen H, Guan Y, Tweardy DJ, Semenza GL, Jing N. "A dual inhibitor that targets HIF-1 α and Stat3 for cancer therapy" (submitted, 2012).

3. Meeting Abstracts.

i. AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics in 11/12-16/2011, at San Francisco, CA (poster attached).



ii. Invited presentation in G-quadruplex, Telomere, and Cancer (GTC-2012) in Academia Sinica, Taipei, 01/5-7, 2012.

Title: G-rich oligonucleotides that targets Stat3 and HIF-1 α for Cancer Therapy
Naijie Jing

Abstract G-rich DNA, which forms G-quadruplex, has been extensively studied as a class of anti-cancer agents. Our group has also developed few G-rich oligonucleotides as novel anti-cancer agents for cancer therapy in last decade.

Signal transducer and activator of transcription 3 (Stat3) has been found to activate in many human cancers (e.g. prostate, breast, lung, head and neck, and pancreatic cancers, etc.). Immune system plays a crucial role in controlling tumor incidence and growth. Stat3 signaling is a major intrinsic pathway of cancer inflammation and mediates the cancer-promoting properties. Stat3 suppresses anti-tumor immune responses and promotes inflammation-induced cancer. Stat3 also participates in oncogenesis through the up regulation of genes encoding anti-apoptosis, cell-cycle regulators, and inducers of angiogenesis, making it as an attractive target for cancer. We have laid the groundwork for development of the G-rich oligonucleotide T40214 that selectively inhibits Stat3 activity *in vitro* and *in vivo*; significant suppression of tumor growth in nude mouse xenografts bearing human breast, head and neck, lung, and prostate tumors.

Hypoxia inducible-factor-1 (HIF-1) plays crucial roles in tumor promotion by up-regulating its target genes, which are involved in energy metabolism, angiogenesis, cell survival, invasion, metastasis, and

drug resistance. The HIF-1 α subunit, which is regulated by O₂-dependent hydroxylation, ubiquitination and degradation, has been identified as an important molecular target for cancer therapy. We have rationally designed JG243 and JG244, which form an intramolecular G-quadruplex, selectively target HIF-1 α and decreased levels of both HIF-1 α and HIF-2 α (IC₅₀ < 2 μ M) and also inhibited the expression of HIF-1-regulated proteins (VEGF, Bcl-2, and Bcl-X_L). JG243 and JG244 dramatically suppressed the growth of prostate, breast, and pancreatic tumor xenografts.

Drug development has moved into targeting molecules based on their important biological function in cancer. A selective inhibition of a target molecule greatly enhances therapeutic efficacy; however, it also results in unexpected consequences, such as development of drug resistance as a single molecule is selectively inhibited; drug becomes less effective or drug responsibility is reduced after prolonged use. It is becoming increasingly evident that increasing the cure rate of many cancers will require combinations of drugs active against more than one validated tumor target. Our recent studies have demonstrated that compared with a single agent blocking activation of either Stat3 or HIF-1 α alone, the combination treatment using T40214 and JG244 to target both Stat3 and HIF-1 α together significantly suppressed growth of human or murine prostate tumors. Also, the combined treatment greatly increased apoptosis in human and murine prostate tumors. The results suggested that combination treatment including a HIF-1 α inhibitor not only improves therapeutic efficacy, but also could reduce the hypoxia-induced drug resistance to other therapies (e.g. T40214) and enhance its drug efficacy.

4. Payment list: Jing N, Guan Y, Twardy, DJ.

FUTURE RESEARCH PLAN

Targeting both HIF-1 α and Stat3 is a pioneer strategy for cancer therapy. Also, no molecule targeting both HIF-1 α and Stat3 has been developed to date. TEL03 targets both p-Stat3 and HIF-1 α under different environments (e.g. normoxia and hypoxia) that could improve tumor response and reduce drug resistance and treatment failure, so that it could improve survival of patients with metastatic disease. In order to move this agent to future clinic study, we designed a three-year approach although the PC093258 grant is only one year left. This approach includes two aims:

Aim1: To determine the drug efficacy of TEL03 in xenografts and PC transgenic mice.

In this aim, we will use nude mice bearing PC tumors and transgenic mice that develop PC to explore several critical issues: (i) determine whether TEL03 has a potent activity and long-term tumor response; (ii) whether TEL03 can increase surviving time and reduce treatment failure in PC therapy. (iii) Enhance delivery TEL03 to pancreas using polyethylene glycol (PEG) conjugation. (iv) To evaluate apoptosis and angiogenesis induced by TEL03 in PC tumors.

Aim2: To determine TEL03 pharmacokinetic (PK) and *in vivo* toxicity data.

PK and *in vivo* toxicity data of TEL03 are very important information for a new drug to move into clinical trials. PK parameters will show the duration of drug activity and the optimal interval time for drug administration. The toxicity studies will determine the LD₅₀ of TEL03 and a long term toxic effect of TEL03. The results will provide critical information for future using TEL03 in clinical treatment. This approach plans to use 180 nude mice and 90 PC transgenic mice.

In next year, which is the last year of PC093258 grant, we plan to focus in Aim1 to determine TEL03 *in vivo* drug efficacy.